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Induced Spawning of the Decapod Crustacean *Sicyonia ingentis*

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Abstract. The dark portion of the light/dark cycle initiated pre-spawning behavior in the penaeid shrimp, *Sicyonia ingentis*. Persistent swimming in the water column (pre-spawning behavior) correlated well with ovulation. Over 96% of the animals that exhibited active swimming behavior were ovulated as determined by the presence of green oviducts and/or their subsequent spawning. More than 87% of the ovulated animals responded to probing of the ovipores, located at the bases of the third pair of pereiopods, by immediately spawning. The ova from probe-induced spawns underwent a normal developmental sequence. A reliable and predictable technique for the assessment of ovulation and acquisition of viable ova from *S. ingentis* is described.

Introduction

Reproductively, decapods can be divided into those that brood their eggs (Pleocyemata) and those that are broadcast spawners (Dendrobranchiata). Present understanding of gamete interaction among the Dendrobranchiata is restricted to a few select species of penaeids (see Clark *et al.*, 1980, 1984). This is largely because researchers must rely on unpredictable natural spawns, a process that is a time consuming and uncertain operation.

Although information is scant, penaeid spawning appears to be correlated with the dark portion of the light/dark cycle. The majority of reports relating to the above are from studies of ovarian maturation and/or larval growth and development; they do little more than mention that gravid females spawned at some time during the night (Kelemec and Smith, 1980; Chamberlain and Lawrence, 1981). The most complete description of spawning concerns *Penaeus japonicus*, which spawns between the hours of 8:00 pm and 12:00 midnight (Hudinaga, 1942). *P. monodon* spawns between 8:00 pm and 6:00 am (Primavera, 1983; Motoh, 1981) and *P. trisulcatus* spawns between 8:30 pm and 10:30 pm (Heldt, 1938).

The penaeid *Sicyonia ingentis* can be inhibited from spawning if maintained under constant light (Griffin *et al.*, 1987); like other penaeids it spawns at night in a dark environment. Anderson *et al.* (1985) suggested that spawning in *S. ingentis* occurs after a period of swimming and that this prespawning behavior may be correlated with ovulation. These suppositions were based upon only a few observations. In the current report we document both the prespawning and spawning behavior of *S. ingentis* and describe a reliable and predictable technique for: (1) assessment of ovulation and, (2) immediate induction of spawning in ovulated animals.

Materials and Methods

Specimens of *Sicyonia ingentis* were collected off the southern California coast in otter trawls during the summer of 1987 and transported to the Bodega Marine Laboratory in chilled (8–10°C) seawater. Gravid females were isolated and placed in a 1000 gallon rectangular glass aquarium supplied with flow-through seawater at ambient temperature (10–14°C). Animals were fed *Artemia salina* and maintained under constant light.

To trigger prespawning behavior, the animals were placed in a dark environment (no lights at night; aquarium covered with black plastic sheet during either late morning or early afternoon). Animals in darkness were monitored with flashlights. To document prespawning and spawning behavior, some animals were allowed to

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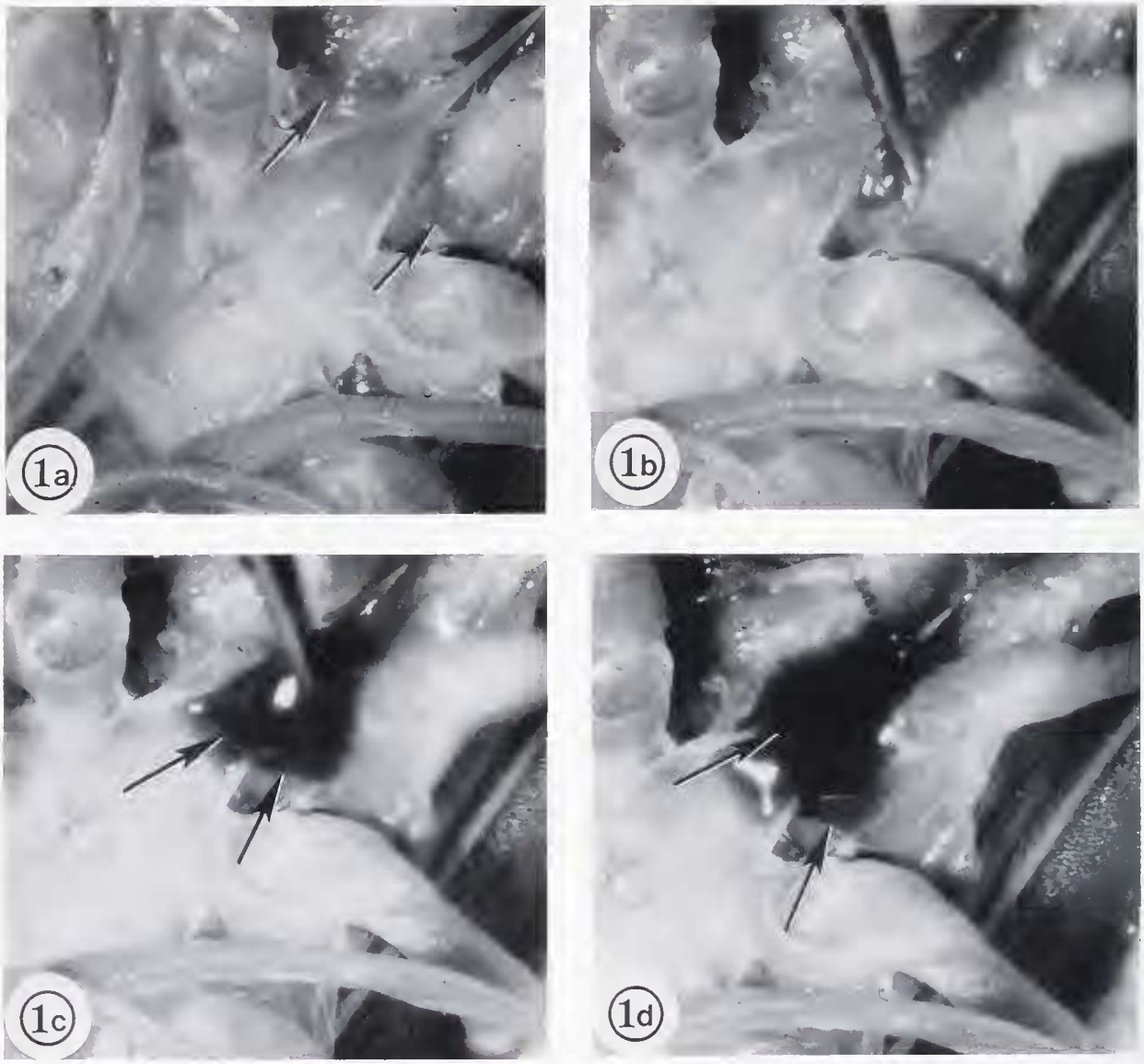

Figure 1. Ventral view of a female *Sicyonia ingentis*. 1a. Ovipores (arrows) located at the bases of the third pereiopods. 1b. The tip of the forceps are at the right ovipore. 1c. Forceps inserted into the right ovipore. The dark patch (arrows) is ova that have been released as a result of opening the ovipore. 1d. Ova have been released (arrows) from both ovipores as a result of opening the right ovipore. (5×)

complete spawning in the aquarium. Others were removed, the coloration of the oviducts was noted, and one ovipore was opened (probed) with forceps. If the probed animal released ova (spawning), she was placed on a 250 ml glass beaker of seawater and observed. We then answered the following questions: (1) did the female continue to spawn?; (2) were ova released from one or both ovipores?; (3) was spawning behavior normal compared with animals that had been allowed to spawn in the aquarium without manipulation?; and (4) were the ova from the probe-induced spawn fertilized? If the probed animal did not release ova, ovarian samples were excised and examined to determine if ovulation had occurred. As a result of ovulation (the loss of follicle cells from the oocytes) ova lay free in the ovary (Anderson *et al.*, 1984). Thus, ovulated and unovulated animals were easily distinguished when the ovary was dissected.

To determine if ova spawned from probed animals had been fertilized, ova were collected in 250 ml glass beakers of seawater and gently agitated for 30 min to keep the cells in suspension, incubated for 3 hours (at 20°C), fixed in seawater buffered 3% glutaraldehyde, and

Table I

Relationship between coloration of oviducts, ovulation, and induction of spawning

	Total number of animals	Green oviducts	Ovulated*	Probe spawns	Normal spawning behavior
Swimmers	65	63	60	57	53
Non-swimmers	28	4	3	2	0

* Ovulation was detected either by subsequent spawning (upon probing) or by dissection of the ovaries.

scored for percent normal cleavage. Fertilized ova undergo a normal (equal) cleavage whereas unfertilized ova exhibit an abnormal (unequal) pattern (Pillai and Clark, 1987). Control animals were allowed to initiate spawning naturally (without being probed) before they were placed on beakers filled with seawater. Percent cleavage in ova from these animals was compared with ova from probe induced spawns.

To determine if probing affected the time between first swimming and spawning, some females that initiated swimming behavior were placed individually in separate rectangular tanks containing flow-through seawater, kept in the dark as described above, and monitored for natural spawning. These animals were checked every 10 minutes for spent ovaries. The time of spawning, if it occurred after transfer to the tanks, was noted. The animals that had not spawned after fifty minutes were removed from the tanks and were probed to induce spawning as described above.

Figure 2. The animal in Figure 1 after being probed at the right ovipore and placed over a 250 ml glass beaker containing seawater. Note the ova being spawned from both ovipores. (0.7×)

Results and Discussion

The manipulation of light cycles dramatically affected spawning in *Sicyonia ingentis*. Within 30–45 minutes after starting a dark cycle animals became active; they repeatedly rose into the water column and fell back to the bottom of the aquarium. Animals that maintained this active swimming behavior eventually remained at the surface and spawned after a variable amount of time. Such swimming activity at night has been described as a prespawning behavior in penaeids (Motoh, 1981; Anderson *et al.*, 1984). The present observations with *S. ingentis* indicate that a dark environment, regardless of the

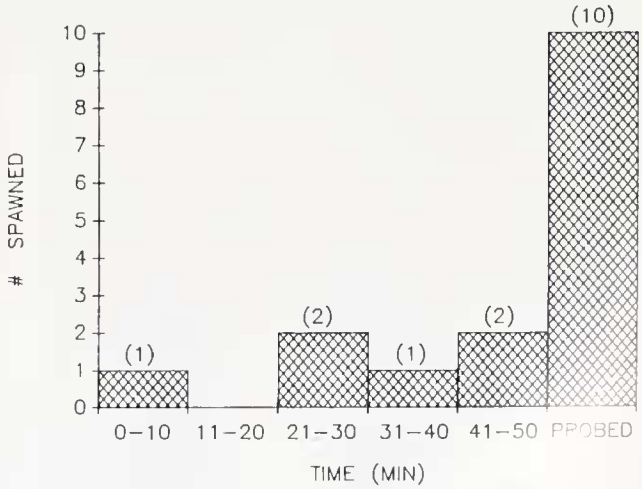

Figure 3. Graph illustrating that natural spawning does not immediately follow ovulation and that the time delay is variable. Animals that had ovulated (those exhibiting prespawning behavior) were placed in individual tanks and monitored at 10 min intervals to determine if spawning had occurred. Those that did not spawn within 50 min were probed. Numbers in parentheses represent the number of animals that spawned during each time period or after probing. Total n = 16.

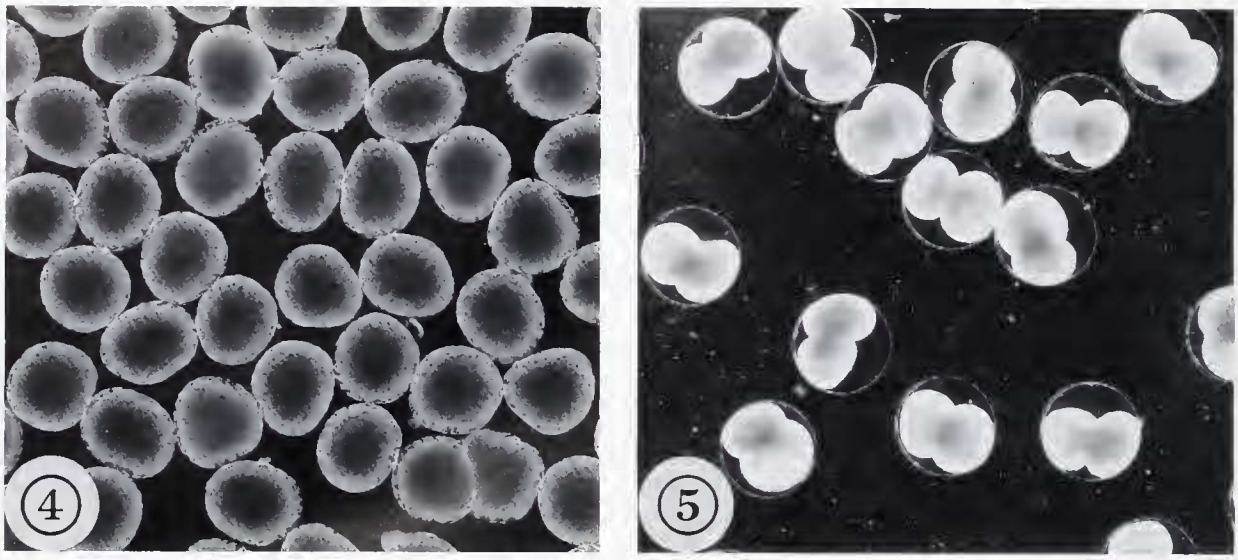

Figure 4. Dark field micrograph of ova from *Sicyonia ingentis* that was induced to spawn by probing at the ovipore. The ova were collected directly into fixative (seawater buffered 3% glutaraldehyde) at spawning. (50×)

Figure 5. Dark field micrograph showing two-cell embryos cultured from an induced spawn. Note the equal cleavage pattern. (50×).

time of the day, initiates prespawning swimming behavior and subsequent spawning.

Spawning behavior in *S. ingentis* is similar to that described for *Penaeus japonicus* (Hudinaga, 1942) and *Penaeus monodon* (Motoh, 1981). During spawning, the animal's pereiopods are held together and projected anteriorly while the pleopods are moved vigorously. Ova are released in a stream from the paired ovipores located at the bases of the third pair of pereiopods and mixed externally with sperm that are ejected from the thelycum (Clark *et al.*, 1984).

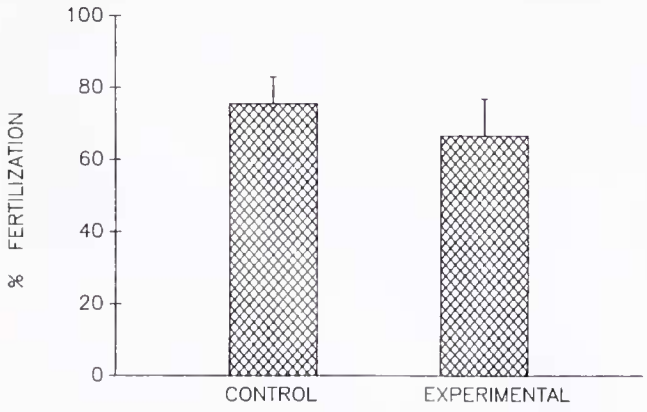

Figure 6. Graph illustrating the percent fertilization and normal development (up to second cleavage) of ova from natural spawns (control) and induced spawns (experimental). The two groups were not significantly different ($P > 0.1$, t test).

Ovulation, which precedes spawning in *S. ingentis* by an unknown period of time (Anderson *et al.*, 1984), correlates well with a change in oviduct color. The oviducts of ovulated females are green; the oviducts of unovulated animals are pale if not indistinguishable. Upon ovulation, free ova move down the oviducts producing a greenish appearance that is visible with the naked eye. Over 96% of the swimmers possessed green oviducts and more than 95% of these animals were ovulated as determined by subsequent spawning (upon probing) or dissection of the ovaries (Table I). Only 4 of 28 (14.3%) animals removed from the bottom of the aquarium (non-swimmers) possessed green oviducts. Two of these animals had completed ovulation and one had partially ovulated (Table I).

Animals that possess green oviducts and exhibit prespawning swimming behavior can be induced to spawn by probing an ovipore (Figs. 1a–d). Over 87% of such animals responded to probing of the ovipores by immediately spawning. More than 92% of this group of animals exhibited normal spawning behavior (Table I) as described above (see Fig. 2). Probing, however, had little effect on animals that did not exhibit pre-spawning behavior (non-swimmers). The only non-swimmers that responded to probing were the two animals that had completed ovulation; however, neither animal exhibited normal spawning behavior nor completed spawning (Table I). These data suggest that two criteria are necessary for successful probe spawns: ovulation and the initiation of prespawning behavior.

Probing one ovipore, regardless of the side of the animal, resulted in release of ova through both ovipores. Probing mechanically opened an ovipore. Although probing experiments demonstrated that spawning can be induced in ovulated swimmers on demand, they did not delineate whether probing decreases the time between ovulation and spawning. Of the 16 ovulated swimmers that were placed in individual tanks and monitored for natural spawning for a period of 50 min, only 6 (37.5%) spawned. The remainder of these animals immediately spawned upon probing (Fig. 3). These results demonstrate that once an animal has undergone ovulation and initiated prespawning behavior, it can be induced to spawn on demand even though the natural time to spawning may be variable.

Induced spawning does not significantly affect zygote quality. Both zygotes from induced and natural spawns were cultured through the first mitotic division (Figs. 4 and 5). Unfertilized ova would have exhibited abnormal (unequal) cleavage in contrast to the normal (equal) cleavage pattern in fertilized ova (Pillai and Clark, 1987). The percent fertilization in the induced spawns (65.5%) was not significantly different from that in natural spawns (75.5%) (Fig. 6). It would not have been unreasonable to expect lower fertilization rates in ova from induced spawns since females induced to spawn released ova prior to being transferred to beakers and initiating normal spawning behavior. Recent evidence indicates that such behavior is required for proper sperm-egg mixing (unpub. data).

This study demonstrates that ovulated animals can easily be selected and induced to spawn on demand under laboratory conditions. It is not known how the mechanical stimulus provided by probing the ovipores induces spawning. Stretch receptors may be involved, but to date this is only speculation. Further studies may explain this phenomenon in more detail. Finally, it is hoped that this technique will advance gamete research with decapod crustaceans.

Acknowledgments

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